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Effects of Continuous Chlorination on Entrained Estuarine Plankton

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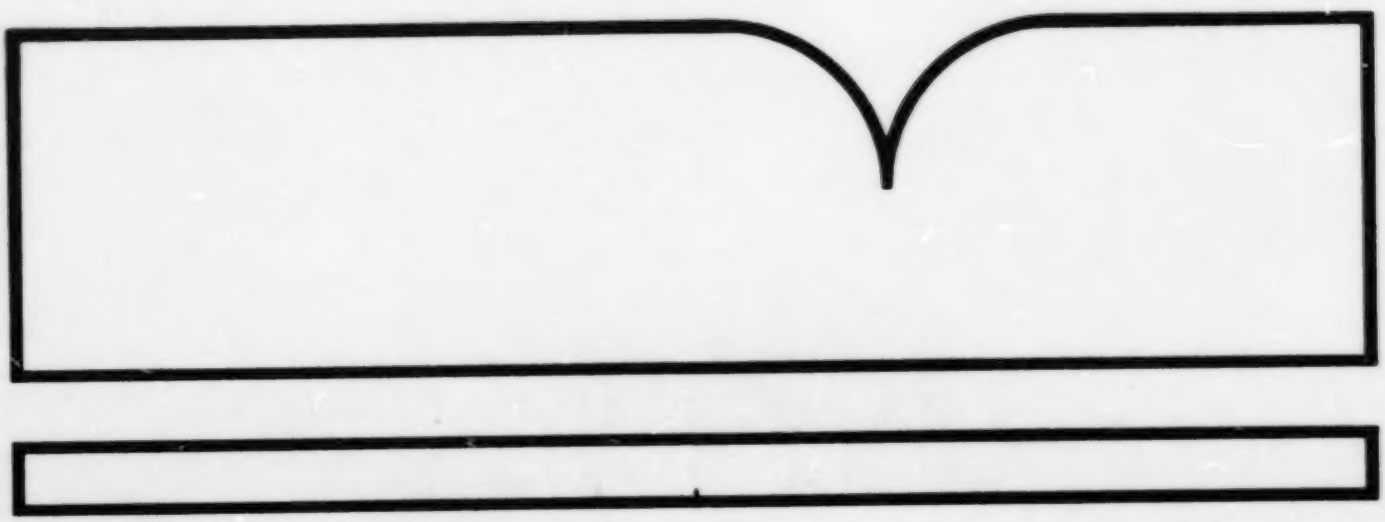

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16. ABSTRACT

The effects of continuous chlorination on entrained plankton are investigated in tests using running sea water and adenosine triphosphate (ATP) as an indicator of biomass. Effects were measured by bioluminescence with the use of luciferin-luciferase reagents from firefly lanterns for the analysis of ATP present in plankton from seawater samples.

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Effects of continuous chlorination on entrained estuarine plankton

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Recent reports indicate that numerous halogenated hydrocarbon by-products are produced during the chlorination process.[1, 2] Some of these compounds are toxic to marine plankton and are thought to persist after the initial chlorination period.

This paper reports the effects of continuous chlorination in running sea water on entrained plankton using the concentration of adenosine triphosphate (ATP) as an indicator of biomass. This measurement was used because ATP is present in all living cells;[3, 4] the concentration is proportional to the living biomass;[5] and dead cells lose ATP rapidly.[6, 7] Three concentrations of sodium hypochlorite (NaOCl) generally considered not toxic to adult fishes and invertebrates were tested in the first system.[8] Higher concentrations were tested in the second systems (Table I). Effects were measured by bioluminescence with the use of luciferin-luciferase reagents from firefly lanterns for the analysis of ATP present in plankton from seawater samples.

Our study began October 8, 1975, and continued for 1 year, giving both immediate and long-term effects of chlorination and its by-products. These effects are, however, inseparable and undefined.

Materials and methods. Estuarine plankton present in flowing seawater aquaria were exposed to NaOCl to simulate constant chlorination. To estimate mortality, ATP from the plankton surviving chlorination was measured and compared as percent of control.

Sea water was pumped from the North Edisto River at Bears Bluff Field Station on Wadmalaw Island, about 40 km (25 miles) south of Charleston, S. C. (lat. 32°40′N, long. 80°18′W), and about 11 km (7 miles) inland from the Atlantic Ocean. Intake sea water was usually turbid and carried considerable filterable particulate material. In a preliminary study, a dry weight of 23 to 37 mg/l was determined. This material, greasy to the touch, is said to be composed of mostly clay and fine silica. The tidal range was 2.0 m (6.4 ft.), with spring tides to 2.3 m (7.6 ft).[9]

The experiments were conducted in two flowing seawater systems originally constructed for use in invertebrate community studies.[10, 11] The present study examined the entrained water only. The larger system, known as Marine Ecosystem Test Unit (METU), was outdoors, subjected to full sunlight, and consisted of ninety-six, 37-l aquaria with a water depth of 20.3 cm. Each received 40 l/h of unfiltered estuarine water from the North Edisto River. Entry of small predatory animals such as fish, shrimp, and crabs was prevented by a 1.6-mm mesh fiberglass screen in the water intake. Entrained planktonic organisms such as eggs, larvae, algae, and juveniles established communities in the aquaria. Escape of free-swimming organisms was prevented by a 1.6-mm mesh screen at the outflow standpipe of each aquarium. Analytical-grade NaOCl was continuously applied with syringe pumps at nominal concentrations of 0, 0.125, 0.25, and 0.5 mg/l at the delivery head. Concentration of residual chlorine in the system was below the detection limit (0.01 mg/l) of the amperometric analyzer used in the study. Each experiment of 32 aquaria had 8 replicates per concentration, randomly distributed through the system. NaOCl solutions were analyzed regularly. The mean of 42 analyses of a 0.125-mg/l stock solution (the only stock solution used) was 0.122 mg/l, standard deviation 0.028. Natural conditions of salinity, light, and temperature were used.

The second system was designed by Hansen[10] as described in his polychlorinated biphenyls (PCB) study. It consisted of four groups of 10 aquaria (44 × 9 × 14 cm), each with a volume of 5.54 l. Water flow rate was 12 l/h. NaOCl was continually added at nom-

TABLE I. Effect of chlorination of running seawater on ATP concentrations, October 1975 to September 1976.

Date	Temp.[a] (C°)	Salinity[a] (g/l)	NaOCl (mg/l) METU 0.0[b]	METU 0.125[c]	METU 0.25[c]	METU 0.50[c]	M System 0.0[b]	M System 0.47[c]	M System 0.94[c]	M System 1.41[c]
Oct. 8	24.3	27.0	0.444	74.0	80.1	62.2				
Oct. 22	24.5	26.7	0.538	77.5	58.9	65.2	0.184	60.3	41.3	32.0
Nov. 6	21.6	27.4	0.335	96.4	45.9	42.3	0.226	54.8	35.8	30.5
Nov. 18	21.2	27.7	0.721	109.5	23.5	32.5	0.156	71.7	44.8	32.6
Dec. 4	16.1	28.7	0.381	125.9	73.2	54.8	0.147	38.7	31.2	18.3
Dec. 17	17.0	28.2	0.179	77.0	73.1	121.7	0.063	90.4	65.0	61.9
Dec. 31	10.1	27.2	0.229	89.5	72.4	56.7	0.262	93.1	47.3	26.7
Jan. 14	11.7	27.0	0.714	80.3	89.7	66.8	0.298	46.9	54.0	24.1
Jan. 28	10.1	26.0	0.716	76.8	47.9	55.8	0.356	63.4	39.7	15.4
Feb. 11	11.8	26.5	0.309	90.6	82.2	59.8				
Feb. 25	17.7	26.5	1.005	74.1	58.7	53.0	0.538	91.4	72.1	69.1
Mar. 10	21.5	26.2	0.584	96.5	92.8	68.3	0.594	87.2	80.1	71.0
Mar. 24	18.1	26.0	0.382	118.5	93.1	89.5	0.374	112.9	62.5	19.5
Apr. 8	22.6	25.8	0.403	74.1	51.6	38.4	0.430	111.6	86.9	69.7
Apr. 21	23.2	27.4	0.401	109.2	114.7	116.7				
May 4	23.2	27.2	0.586	85.0	53.2	65.0				
May 19	25.1	27.6	0.715	42.0	45.3	41.3	0.410	68.2	60.0	24.1
June 2	25.2	27.1	0.615	64.5	65.8	52.8	0.628	74.8	81.8	83.7
June 16	25.4	25.5	0.521	90.4	63.5	44.9	0.556	32.7	66.5	90.2
June 30	27.3	24.6	0.752	54.5	74.3	71.6	0.372	50.2	33.8	32.7
July 14	29.2	21.9	0.550	100.0	113.2	82.7	0.438	73.0	63.4	55.2
July 29	30.8	20.9	0.970	83.5	100.7	57.2	0.656	72.5	63.4	36.5
Aug. 11	29.5	23.8	0.920	97.3	84.6	64.5	1.010	88.3	56.5	42.3
Aug. 25	28.6	26.6	0.570	87.0	89.8	82.8				
Sept. 9	30.4	28.3	0.553	73.2	95.4	90.7	0.538	78.8	54.6	17.2
Sept. 22	25.8	28.1	0.116	118.1	173.2	100.0	0.153	104.5	49.0	40.5
Mean	23.0	26.4	0.55	87.1	77.6	66.8	0.40	74.5	56.7	42.5
Standard deviation			0.36	19.6	29.5	22.7	0.22	22.5	15.9	23.1

[a] Mean values for each 2-week sampling period.
[b] In μg ATP per litre.
[c] Percentages of control concentrations.

inal levels of 0.0, 0.47, 0.94, and 1.41 mg/l to each of four groups of 10 aquaria. Since the container sizes of this system were relatively miniature, it is hereafter referred to as the M system. In contrast to the METU, the M system was located indoors and illuminated by low-level fluorescent bulbs that were not intense enough to encourage growth of macroalgae in the aquaria. Additionally, the mean water exchange rate was 2.16 times per hour (2.3 hours required for 99% partical replacement of water in aquaria) or twice that of the METU.

Samples were collected from both systems on a 2-week schedule from October 8, 1975, to September 22, 1976 (Table I). In the METU, 500-ml samples were collected by pooling equal amounts of water from the eight aquaria at each chlorination level. Because of its structure, each chlorination concentration level could be sampled twice. In the M system, each chlorination level involved a single chamber, and only single samples were taken. Samples were passed through a 0.110-mm nylon screen immediately after collection to remove larger organisms. Then they were thoroughly mixed and 25 ml of sample was filtered under vacuum through a 0.22-μ, 25-mm diam membrane filter. The filter and its ATP-containing microorganisms were extracted 5 minutes in 5 ml of hot (98°–98°C) 0.01 *M* morpholinopropane sulfonic acid buffer, pH 7.4, to which had been added 0.01 *M* magnesium sulfate ($MgSO_4$) and 0.001 *M* sodium

ethylenediamine tetraacetic acid (EDTA). Samples were analyzed immediately or stored at −20°C for later analysis.

Analyses were performed with a highly sensitive photometer reading directly in femtograms (10^{-15} g). Luciferin-luciferase firefly enzyme substrate powder was obtained (Du Pont Instrument Products Division) and prepared according to detailed instructions.[12] It was dispensed in 0.1-ml volumes by automatic pipette into 5 mm × 50 mm disposable cuvettes at least 30 minutes prior to use to allow attenuation of the inherent luminescence of the material. Analyses were conducted at 20°C ± 1°C. Standardization was performed with solutions of analytical-grade adenosine-5-triphosphate, dipotassium. Ten microlitres of ATP standards or extracted field samples were injected into the reaction chamber with a 50-μl syringe, using an automatic injection device as described in the Du Pont instruction manual.[12]

Results and discussion. The results of this controlled running sea water study of chlorination by measuring ATP are shown in Table I. Of 292 samples analyzed, 3 were rejected as sampling errors. In each rejected sample, the ATP concentration was 2.4 to 10 times the control sample for that date.

The aquaria systems operated continuously for 1 year, permitting examination of the effects of continuous chlorination at concentrations below those generally considered effective for the control of pathogens and fouling organisms. Temperature, salinity, and pH were dependent on natural conditions. Size of organisms allowed to enter, time of experiments, and chlorination were controlled.

Chlorination reduced the microbial biomass of sea water at all concentrations tested. In the METU, the mean control ATP concentration for 52 weeks was 0.55 μg/l. In aquaria treated with NaOCl, 0.125 mg/l reduced ATP to 87.1% of the control concentration, 0.25 mg/l to 77.6%, and 0.5 mg/l to 66.8%. In the M system, 0.47 mg/l reduced ATP to 74.5% of the control concentration, 0.94 mg/l to 56.7%, and 1.41 mg/l to 42.5%.

One common dosage level was present in each system for comparing the effects of chlorination (0.5 mg/l in the METU and 0.47 mg/l in the M system). Mean control ATP in the METU (0.55 μg/l) was slightly higher than the M system (0.40 μg/l). Mean ATP for the METU was 66.8% of control, compared with 74.5% of control in the M system.

In the METU, extensive growth of surface algae was present in response to natural lighting. The control aquaria contained abundant growth of net-like filamentous diatoms (*Melosira*) but little or none of green algae. Fouling organisms grew in the passages of the water distribution system. At an NaOCl concentration of 0.125 mg/l, algae in the aquaria were a mixture of filamentous diatoms and green algae (including *Enteromorpha* and *Cladophora*), with some fouling growth in the water passages. At 0.25 and 0.5 mg/l, no fouling occurred in the water passages and filamentous diatom growth was replaced by mats of green algae. In the water column, populations of swimming amphipods were observed at all levels of chlorination. In the M system, little algal growth was present and swimming amphipods were rarely observed. These differences were, perhaps, in response to a higher mean water exchange rate in the M system and the differences in light available for algal growth.

Conclusions. Field studies by Gentile *et al.*[13] support use of the measurement of ATP as a means of evaluating damage done to planktonic organisms by chlorination. Residual chlorine concentrations of < 1.0 mg/l were examined in a study of the Morgantown Generating Station of the Potomac Electric Power Co. They concluded that concentrations of 0.32 and 0.55 mg/l chlorine resulted in irreversible loss of living biomass. In this study, nominal NaOCl concentrations of 0.125 to 1.41 mg/l resulted in a reduction in ATP present in sea water.

Further studies of the effects of low-concentration, continuous chlorination would seem to be in order. More information is needed on specific species of organisms and on the effects on the various life stages. This study allowed examination of continuous chlorination at concentrations that did not produce a measurable residual. They did, however, result in a measurable reduction of microbial biomass as measured by concentration of ATP in estuarine sea water.

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REFERENCES

1. Jolley, R. L., *et al.*, "Chlorination of Organics in Cooling Waters and Process Effluents." In "Proceedings of the Conference on the Environmental Impact of Water Chlorination." R. L. Jolley [Ed.], Natl. Inf. Service, U. S. Dept. of Commerce, Springfield, Va., Conf-751096, 115 (July 1976).
2. Glaze, W. H., *et al.*, "Analysis of New Chlorinated Organic Compounds Formed by Chlorination of Municipal Wastewater." In "Proceedings of the Conference on the Environmental Impact of Water Chlorination." R. L. Jolley [Ed.], Natl. Inf. Service, U. S. Dept. of Commerce, Springfield, Va., Conf-751096, 153 (July 1976).
3. Strehler, B. L., and McElroy, W. D., "Assay of Adenosine Triphosphate." In "Methods in Enzymology," Vol. 3. S. P. Colowick and N. O. Kaplan [Eds.], Academic Press, New York, N. Y., 871 (1957).
4. Huennekens, F. M., and Whitely, H. R., "Phosphoric Acid Anhydrides and Other Energy-rich Compounds." In "Comparative Biochemistry," Vol. 1. M. Florkin and H. S. Mason [Eds.], Academic Press, New York, N. Y., 129 (1960).
5. Seligar, H. H., and McElroy, W. D., "Spectral Emission and Quantum Yield of Firefly Bioluminescence." *Arch. Biochem. Biophys.*, **88**, 136 (1960).
6. Holm-Hansen, O., and Booth, C. R., "The Measurement of Adenosine Triphosphate in the Ocean and its Ecological Significance." *Limnol. & Oceanog.*, **11**, 510 (1966).
7. Hamilton, R. D., and Holm-Hansen, O., "Adenosine Triphosphate Content of Marine Bacteria." *Limnol. & Oceanog.*, **12**, 319 (1967).
8. Davis, W. P., and Middaugh, D. P., "A Revised Review of the Impact of Chlorination Processes upon Marine Ecosystems: Update 1977." "Water Chlorination: Environmental Impact and Health Effects." Vol. 1. Ann Arbor Science, Ann Arbor, Mich., 283 (1977).
9. "Tide Tables of the East Coast of North and South America including Greenland." U. S. Department of Commerce, NOAA, 96 (1975).
10. Hansen, D. J., "Aroclor® 1254: Effects on Composition of Developing Estuarine Animal Communities in the Laboratory." *Contributions in Marine Science*, **18**, 18 (1974).
11. Davis, W. P., *et al.*, "Marine Ecosystem Testing Units: Design for Assessment of Benthic Organism Responses to Low-Level Pollutants. *Helgoländer wiss. Meeresunters* (Ger.), **30**, 673 (1977).
12. "Instruction Manual 760 Luminescence Biometer." E. I. duPont De Nemours & Co., Inc., Wilmington, Del. (1970).
13. Gentile, J. H., *et al.*, "Power Plants, Chlorine and Estuaries." U. S. Environmental Protection Agency, EPA-600/3-76-055 (1976).